

PROJECT TITLE : NITRATE-REDUCTION BY CONTROLLED FERMENTATION  
PERIOD COVERED : JUNE 21 - JULY 20  
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1. TRIALS

1.1. LEAR trial 7

Several tentatives were made to improve the denitrification of strip extract during LEAR trial 7. This trial was extended to 4 weeks, and included cooling of the fermenter at each week-end (1). First of all, the pH of the extractor feedwater was lowered in order to avoid any microbial growth causing nitrite formation (2). With the continuous addition of 3% of phosphoric acid at 10% concentration, no nitrites were formed during the extraction. Later on, some trace elements (Mo, Zn, Se) were added into the fermenter but they did not improve the efficiency of the denitrification, which remained uncomplete above a dilution rate of  $0.1 \text{ h}^{-1}$ . During the fourth week of the trial however, it was possible to run the process much better, after modification of the strip : water ratio of the extraction from 1 : 10 to 1 : 15. It is almost certain that a new sugar:nitrogen ratio will have to be determined for strip extracts, since an excess of sugar can have a blocking effect on the respiration of the yeasts.

1.2. LEAR trial 8

LEAR trial 8 has been running in the 20-l fermenter since July 15. Its objective is to determine the exact amount of sugar needed by the yeasts for the denitrification of a strip extract. An extraction was carried out in the Rotocell with a strip : water ratio of 1 : 15 and the extract was sterilized and stored as feedstock for the 20-l fermenter (3). The sugar solution was fed separately into the fermenter by means of a metering pump which allowed accurate control of the flow-rate. The fermenter was continuously run for 110 hours at various dilution rates : 0.08, 0.14, 0.18 and  $0.21 \text{ h}^{-1}$  and total denitrification was achieved all the time. The sugar:nitrogen ratio was about 24 g of 100% dextrose for 1 g of nitrogen ( $\text{NH}_3\text{-N}$  and  $\text{NO}_3\text{-N}$ ) and the acid consumption was 2% of the flow feeding the fermenter. After 22 hours at a dilution rate of 0.21, no more yeasts were found in the fermenter. They had been washed away by the dilution rate because they did not grow fast enough. However, no nitrates were detected in the extract at that time, because the denitrification was probably achieved by other micro-organisms which grew faster in these conditions. At this stage, the fermentation was stopped and will be re-started with a new batch on July 21.

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2. NITROSAMINES IN LEAR EXTRACTS

A series of LEAR extracts was given to the Research laboratory for analyses of nitrosamines. Although no volatile nitrosamines were detected, some relatively high concentrations of NNN, NATB and NNK were found in these extracts (4). Therefore more detailed investigations will be conducted involving samples of extracts before and after sterilization and tobaccos.

3. REFERENCES

1. Ruf-C Monthly Report, June 1981
2. Lüthi-N Monthly Report, June 1981
3. Lüthi-N Memo : Essai LEAR 8, July 14, 1981
4. Fink-W Memo to Ruf-C : Nitrosamines in LEAR, June 22, 1981



CLR/sde/JULY 28 1981

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